PROGRESS REPORT

TO THE

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

Research Grant NGR-05-020-137: Structure and Function of Proteins and Nucleic Acid

Semi-annual Report for the Period July 1, 1966 to December 31, 1966

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1. Publications

a. Stryer, L., Excited-State Proton-Transfer Reactions. A Deuterium Isotope Effect on Fluorescence, J. Am. Chem. Soc. 88, 5708 (1966).

b. Haugland, R. P., Stryer, L., Stengle, T. R. and Baldeschwieler, J. D., NMR Studies of Antibody-Hapten Interactions Using a Chloride Ion Probe, Biochemistry 6, 498 (1967).

c. Hundley, L., Coburn, T., Garwin, E., and Stryer, L., A Nanosecond Fluorimeter, Rev. Sci. Instrum. 38, 488 (1967).

d. Stryer, L., Holmgren, A., and Reichard, P., Thioredoxin. A Localized Conformational Change Accompanying Reduction of the Protein to the Sulfhydryl Form., Biochemistry 6, in press (1967).

3. Haugland, R. P., and Stryer, L., A Fluorescent Probe at the Active Site of α-Chymotrypsin, in International Symposium on Conformation of Biopolymers, in press (1967).

2. Nanosecond fluorescence spectroscopy

A new type of nanosecond fluorimeter suitable for measuring the emission kinetics of chromophores which have excited state lifetimes of 1 nsec or longer has been constructed. The apparatus is novel in two respects: (a) the acquisition and processing of the nanosecond data are accomplished in entirety by computers; (b) the pulsed light source is an oxygen spark-gap lamp operated in a relaxation mode. The lamp exhibits high intensity over a broad spectral range as well as a short pulse duration. In the wavelength interval from 230 to 470 mμ, there were 6×10^{11} photons per pulse at a repetition rate of 2.1 kHz. The spectrum of the lamp was approximately flat between 200 and 600 mμ. The rise and fall times of the light pulse were 0.7 nsec and 1.1 nsec. The accompanying current pulse had rise and fall times shorter than 0.43 nsec. A 1P21 photomultiplier tube was used as the detector in the fluorimeter. The output of the 1P21 was connected to a sampling oscilloscope that was triggered by a synchronous current pulse from the light source. The analog output of the sampling oscilloscope was digitized, stored, and averaged on a LINC computer, and then transferred to magnetic tape for processing on an IBM 7090. The

apparatus has been used in studies of the rotational mobility of biological macromolecules.

3. A fluorescent probe at the active site of chymotrypsin

A highly fluorescent chromophore has been specifically inserted at the active site of α-chymotrypsin. A spectrofluorimetric study of this modified chymotrypsin has provided detailed information concerning the active site of the enzyme. The fluorescent reagent is the p-nitrophenyl ester of anthranilic acid which reacts with the serine residue at the active site to form a stable anthraniloyl acyl enzyme, which is enzymatically inactive. Chymotrypsinogen, di-isopropyl-phosphoryl chymotrypsin, trypsinogen, lysozyme, and serum albumin do not react at all, while trypsin reacts slowly.

The anthraniloyl group can be selectively excited since its absorption and emission maxima are distinct from those of the aromatic residues of the protein. The absorption and emission spectra of the anthraniloyl chromophore vary with solvent polarity, thereby making it feasible to determine the polarity of the immediate environment of the acyl group in the enzyme. It is concluded that the environment of the anthraniloyl group at the active site is highly polar. The fluorescence polarization and emission kinetics in the nanosecond range were measured to determine the flexibility of the active site. The rotational relaxation time of the anthraniloyl group is 49 nsec, indicating that the active site of the acyl enzyme is rigid. The anthraniloyl chromophore has no rotational mobility independent of the whole chymotrypsin molecule.

4. Nuclear magnetic resonance studies of antibody-hapten interactions

The interaction of a hapten with antibody was investigated by a new technique: ^{35}Cl NMR spectroscopy, using a mercury-labeled hapten. The hapten, 2,4-dinitro-4'-(chloromercuri)diphenyl-amine, binds strongly to antidinitrophenyl antibody in 1M NaCl. The binding of chloride ions by the mercury atom of the hapten-antibody complex and the rapid exchange of the bound chlorine with chloride ions in the solvent produce a broadening of the ^{35}Cl NMR resonance at protein concentrations as low as 10^{-6} M. The NMR spectra provide information concerning three facets of the hapten-antibody interaction: (a) accessibility of the mercury atom in the hapten-

antibody complex to chloride ion in the solvent; (b) binding affinity and stoichiometry of the hapten-antibody complex; and (c) rotational mobility of the bound hapten. It seems likely that the active sites of a variety of proteins can be similarly studied by ^{35}Cl NMR spectroscopy using substrates which contain a covalently bonded mercury atom.

5. Potential applications in planetary explorations

As indicated in our previous progress report, several aspects of our research may prove valuable in planetary explorations. The nanosecond fluorescence apparatus is particularly significant for two reasons. The acquisition and processing of the experimental data are accomplished in entirety by computers. This means of handling data is obviously relevant to the problem of the automated biological laboratory. Second, the use of fast time-resolution enhances the sensitivity of fluorimetry as a method of analysis of samples. We are continuing our work on selective fluorescent labeling reagents, as illustrated by the fluorescent probe at the active site of chymotrypsin.

May 1, 1967